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"IN SEARCH OF BRASSICA GERMPLASM IN SALINE SEMI-ARID AND ARID REGIONS OF INDIA AND PAKISTAN FOR RECLAMATION OF SELENIUM LADEN SOILS IN USA"

Attached is the above titled paper.

This paper provides an excellent source of new information and background for personnel involved with saline agriculture. It is an outstanding example of NRCS and ARS working together.

Prepared by David Dyer, Manager, Lockeford Plant Materials Center, Lockeford, California

In search of *Brassica* germplasm in saline semi-arid and arid regions of India and Pakistan for reclamation of selenium laden soils in USA

G.S. Bañuelos[1], D. Dyer[2], R. Ahmad[3], S. Ismail[3], R.N. Raut[4], and J.C. Dagar[5]

[1]Water Management Research Laboratory, USDA-ARS, Fresno, California 93727
[2]Plant Materials Center USDA-SCS, Lockeford, California 95237
[3]Biosalinity Laboratory, University of Karachi, Karachi, Pakistan
[4]Division of Genetics, Indian Agricultural Research Institute, New Delhi, India
[5]Central Soil Salinity Research Institute, Karnal, India

Abstract
High concentrations of selenium (Se) found in some saline soils may be detrimental to sustainable agriculture in parts of the western USA. Remediation strategy with *Brassica* species may be practical to reduce soil concentrations of Se to non-toxic levels. Collecting germplasm native to or growing in semi-arid and arid saline regions like Pakistan and India, may provide Californian and Western US growers with additional plant materials to evaluate Se removal by plant uptake in saline/Se contaminated soils.

California is one of the leading agricultural producers in the world today mainly because of its climate and extensive and developed irrigation network in central California. Many of the problems associated with the transformation and mobility of trace elements like Se are a result of excessive irrigation on sedimentary sandstones and shale from the California Coastal Ranges (27). The presence of natural clay barriers in the soil profile have caused formation and enlargement of shallow groundwater bodies that inhibit the transport of salts from the root zone. Thus, salts and trace elements tend to accumulate by evaporation because surface and subsurface drainage in many irrigated basins is slow to reach natural outlets (i.e., rivers). With little precipitation and arid climate in central California, salinity increases throughout the root zone with ultimately alkali deposits forming on the soil surface.

Cultivation of crops in salt affected soils requires specialized approaches: including the installation of subsurface tile drains and selecting adaptable plant species for the saline environments. Although leaching salts from the root zone is necessary for sustainable agronomic production, it is the prevalent cause for the migration of Se into groundwater and drainage effluent (23). Some of these saline agricultural drain waters with a specific conductances (a salinity parameter) ranging from 0.4 dS m^{-1} to 68 dS m^{-1} [surveyed by U.S. Geological Survey (8)] contained ranges of Se from 84 to 4,200 mg L^{-1} [water containing >1000 μg L^{-1} is considered toxic (10)]. Some of these waters have been used in wildlife habitats where serious biological effects from elevated levels of Se have been documented in central California (20, 23, 24).

Emerging evidence indicates that at least 10 other sites in the western US are contaminated with Se and similar toxicological problems are anticipated (i.e., mutations of waterfowl). Studies have been conducted in California soils particularly susceptible to Se (34), to investigate strategies for lowering levels of Se entering the agricultural ecosystem by utilizing efficient irrigation delivery systems (22), developing drainage water management (1, 2), and evaluating vegetative management as a form of Se remediation (7). Plants might specifically be grown to maximize Se accumulation in aerial organs which could be harvested, removed from the site, and disposed of elsewhere. Combining irrigation and drainage technology with the incorporation of selected plants on problematic soils may reduce the amount of Se-laden effluent for disposal, minimize deep percolation losses, reduce water application depth, and ultimately limit the amount of Se entering the groundwater (7). If levels of soil Se are not reduced in soils where agronomic or forage crops are grown, its increased solubility, mobility, and eventual movement into groundwater may lead to additional Se poisonings of waterfowl and other animals in California (20) and in the north eastern region of Punjab, India (9, 13), respectively.

The Se content of soils has received considerable attention in many countries, mainly those where the role of Se in animal health has been widely recognized. Some soils in the US, Mexico, Ireland, Israel, Australia, and Haryana, India (29) contain high total water soluble Se, from which crops may absorb sufficient Se to inhibit plant metabolism. During chemical weathering of rocks; Se is easily oxidized and the state of its oxidation and solubility are controlled by the oxidation-reduction regime and pH of the environment. Selenium has four oxidation states: Selenide (Se^{-2}), elemental Se (Se^{0}), selenite (SeO_3^{-2}), and selenate (SeO_4^{-2}). Selenate is most stable, abundant, mobile, and available to plants in alkaline systems. Since Se is not known to be a necessary micronutrient for plant nutrition, the element probably enters the plant as a selenate salt with the same uptake mechanism as SO_4 (17). Presumably a higher Se absorption would then occur in crops that accumulate large quantities of sulfur (S) (i.e., *Cruciferae*) compared to other plants grown in seleniferous areas (14). In this regard, Bañuelos and Meek demonstrated that S accumulating vegetables like broccoli and cabbage were capable of accumulating Se (selenate) to exceedingly high concentrations (Table 1) (4). Because of both the high requirement of S in *Cruciferae* and the chemical and physical similarity between Se and S, isolated enzyme systems in species within the *Cruciferae* may be able to utilize S and Se metabolites interchangeably and concentrate high amount of Se. Species, that tolerate high concentrations of S, may not completely discriminate between Se and S salts (4, 14) and accumulate high amounts of available Se.

Usually, plant species amassing high Se concentrations are known as "Se accumulators" (25). These plants are capable of growing in seleniferous soils without exhibiting apparent phytotoxicities. Recently Bañuelos and Meek, Wu et al., Parker et al., and Watson reported on the ability of different plant species (i.e., *Brassica* spp., *Festuca*, *Atriplex*, and *Astragalus* spp.) to take up Se (5, 21, 35, 36). In comparison to the other species, *Brassica juncea* appeared to be one of the most effective absorbers of Se in non-saline conditions (Table 2), and somewhat less effective in saline conditions (Table 3). Additional preliminary studies by Bañuelos and co-workers indicate that *B. juncea* volatilizes a portion of the absorbed Se to a gaseous Se compound. The methylation of Se may be Se detoxification mechanism developed by the plant to lower tissue concentrations of Se (18). Hypothetically it may then be possible to use plant volatilization as an additional dispersal pathway for excessive Se concentrations. Plant removal of Se by uptake and volatilization may be more feasible than physically removing contaminated soil or taking seleniferous regions out of crop production. It is far easier to use Se accumulating species such as *Brassica* to remove Se from the soil, and then safely dispose of harvested vegetation in bio-mass energy cogeneration plants or by blending carefully in animal forage than physically isolating a contaminated (otherwise unproductive) soil through covering, excavation, and/or removal.

Brassica species present enormous diversity in forms such as vegetable, oil, fodder, and condiments as end products. The different forms of *Brassicas* in India comprise three ecotypes of 20 chromosomes: *B. campestris* ssp. oleifera (yellow sarson, brown sarson, and toria, collectively called rape) and 36-chromosome *B. juncea*. *Brassica juncea* is similar in its general characteristics as an oilseed to rape (*B. napus*), but has the reputation of possessing agronomic advantages over rape, notably greater resistance to drought, shattering and disease (16). *Brassica juncea* is grown sparingly in many arid and semi-arid countries for the production of condiment mustard, but it is also grown on a large scale on the Indian sub-continent (Fig. 1) and in Pakistan (Fig. 2) for the production of vegetable oil (15). In addition, Indian mustard is used as green supplemental fodder for animals, and its seed cake is also fed to animals.

To cope with the limitations of land and water resources for agriculture, and the inability (financially) of many typical Pakistani and Indian growers to employ innovative irrigation and drainage water management in saline soils, cultivating salt-tolerant *Brassica* species is common. Many wild cultivars have been domesticated for agronomic purposes for cultivation in normally unproductive saline soils. Since enormous genetic variability exists within each species of *Brassica* (in the form of sub-species and ecotypes) *Brassica* germplasm from different saline soil sites within Pakistan and India may be useful for vegetation for removing Se from saline soils of the central California. Reclamation with *Brassica* species in problematic California soils requires plants to have relatively good salt tolerance because of the oftentimes strong correlation between Se levels and soil salinity (12). Information is limited available regarding salt tolerance of *Brassica* species (19). For this reason, United States Department of Agriculture (USDA) Agencies such as the Soil Conservation Service (SCS), Agricultural Research Service (ARS), and the Office of International Cooperation and

Development (OICD) support research to collect indigenous or synthesized salt tolerant ecotypes of *Brassica* species found in Pakistan and India that may be used to help remove Se from saline soils. India would be a logical place to collect materials since production of mustard in India is larger than in any other country (28). *Brassica* species collected from different regions or obtained from germplasm seed banks in Pakistan and India are presently being evaluated by the USDA-ARS and the USDA-SCS for ability to tolerate high saline and Se conditions in central California. In conjunction with Se uptake, cultivars are evaluated for ability to volatilize Se as a gaseous compound (18). Once Se is methylated by the plant, the gaseous compound is released into the atmosphere, diluted and dispersed by air currents directly from the contaminated source. Dimethylselenide is 500-700 times less toxic than aqueous selenite or selenate (11). This process permanently reduces the soil Se levels.

Greenhouse studies initially help determine *Brassica* cultivars to be planted in subsequent experiments for removal of Se from saline soils of central California. Field evaluation of non-native ecotypes of *B. juncea* in the central California requires caution because the planting may modify vegetation in isolated areas and influence insect patterns. If damaging levels of insects (i.e., beet leafhopper) occur on these plantings, control measures, including multiple harvests of mustard plantings might be necessary to decrease potential population densities and reproductive capacities of insects. All tested cultivars need close monitoring to insure they do not exhibit disease, insect, and weed problems, or attract certain types of wildlife. Moreover, information is needed about disposal/use of plants that accumulate high concentrations of Se. Watson (35) and Bañuelos et al. (3) suggested using plants as potential sources of Se for animal feed in Se deficient regions. This concept implies carefully blending dried, harvested plant material with livestock feed or adding harvested plant material to soils in Se deficient soils where pasture or forage is produced. This method of disposal may help animals meet their necessary requirement of Se (33) (selenium deficiency is for the livestock industry is as serious a problem as Se toxicity).

Conclusion

Many of the *Brassica* cultivars used in the USA have a narrow genetic base. Evaluating different mustard species from India and Pakistan with more genetic diversity may provide growers in California and India with invaluable *Brassica* cultivars for reclamation of Se laden soils. This could mean discovery of new *Brassica* crops or new genes for salt tolerance and Se absorption/volatilization. Combinations of using water management practices (i.e., improved irrigation efficiency and uniformity) and salt-tolerant *Brassica* ecotypes with the ability to accumulate Se for particular sites could reduce detrimental effects that irrigated agriculture exerts on the agro-environment in areas of Se problematic soils. Meeting these requirements in arid and semi-arid environments might include production of *Brassica* species for Se contaminated regions.

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Table 1. Selenium concentrations in vegetables grown in selenium treated soil (4).

Vegetable species	Plant part	Se concentration[a] Selenite	Selenate
		---- mg/kg^{-1} dry weight ----	
Swiss chard			
(*Beta vulgaris*	Mid-rib	30	115
var. *cicla*)	Leaf	12	750
Collards			
(*Brassica oleracea*	Mid-rib	23	350
var. *acephala*)	Leaf	38	235
Cabbage			
(*Brassica oleracea*	Old leaves	38	260
var. *capitata*)	Young leaves	65	450
Broccoli			
(*Brassica oleracea*	Floret	200	1,200
var. *botrytis*)	Leaves	50	370

[a]Vegetables irrigated with water containing selenite or selenate to raise the concentration of Se to 5 mg kg^{-1} soil.

Table 2. Effect of plant species on removal of Se from soil treated with selenite or selenate before planting[a] (7).

Plant	Selenite			Selenate		
	Se in:		Relative amount of applied Se not found in soil/plant	Se in:		Relative amount of applied Se not found in soil/plant
	Soil	Plant		Soil	Plant	
	---- μg Se/pot^{-1} ----		%	----- μg Se/pot^{-1} -----		%
Milkvetch (*Astragalus lentiformis*)	2,850[b]	75	16	1,750	880	25
Black mustard (*Brassica nigra*)	3,050	85	10	2,100	890	15
Indian mustard (*Brassica juncea*)	3,000	400	3	800	2,500	6
Australian saltbush (*Atriplex nummularia*)	2,950	85	13	1,650	1,640	6
Saltbush (*Atriplex semibaccata*)	3,199	70	9	1,800	875	24
Tall Fescue grass (*Festuca arundinacea*)	3,050	60	11	2,300	580	18

[a]One kg soil in each pot was treated with 3,500 μg Se as selenate or selenite in solution added before planting.
[b]Values presented are the means from ten replications.

Table 3. Mineral composition in shoots and roots of Indian mustard grown hydroponically at different levels of salt, Se, and B (6).

Treatment: Salt[a] (dS/m^{-1})	Se (mg/L^{-1})	B (mg/L^{-1})	[Se] Shoots (mg/kg^{-1} dry wt)	[Se] Roots (mg/kg^{-1} dry wt)	[B] Shoots (mg/kg^{-1} dry wt)	[B] Roots (mg/kg^{-1} dry wt)
0.5	0	0.1	8(34)[b]	3(23)	<1	<1
3.0	2	3	181(21)	46(26)	153(8)	50(21)
3.0	6	3	351(17)	209(24)	137(12)	42(29)
3.0	15	3	1108(16)	554(18)	118(21)	39(35)
10.0	2	3	97(25)	65(12)	121(15)	46(24)
10.0	2	10	68(25)	57(22)	185(12)	66(18)
10.0	2	15	57(22)	52(14)	207(15)	78(8)
10.0	6	3	272(10)	179(15)	101(22)	42(31)
10.0	6	10	344(17)	283(19)	181(10)	72(13)
10.0	15	3	1026(12)	395(13)	88(11)	44(38)
10.0	15	10	862(11)	435(11)	145(11)	83(20)
15.0	2	10	62(20)	48(19)	108(17)	57(26)
15.0	2	15	54(14)	36(21)	129(21)	154(12)
15.0	15	15	1300(11)	553(12)	160(11)	214(21)

[a]Synthetic water culture solution contained the following salts: $MgSO_4 \cdot 7H_2O$, Na_2SO_4, NaCl, $Ca(NO_3)_2 \cdot 4H_2O$, K_2HPO_4; additional salinity was increased with NaCl and $CaCl_2$ on approximately 5:1 ratio by weight.

[b]Values are presented as means from 30 replications followed by the coefficient of variation expressed as percentages.

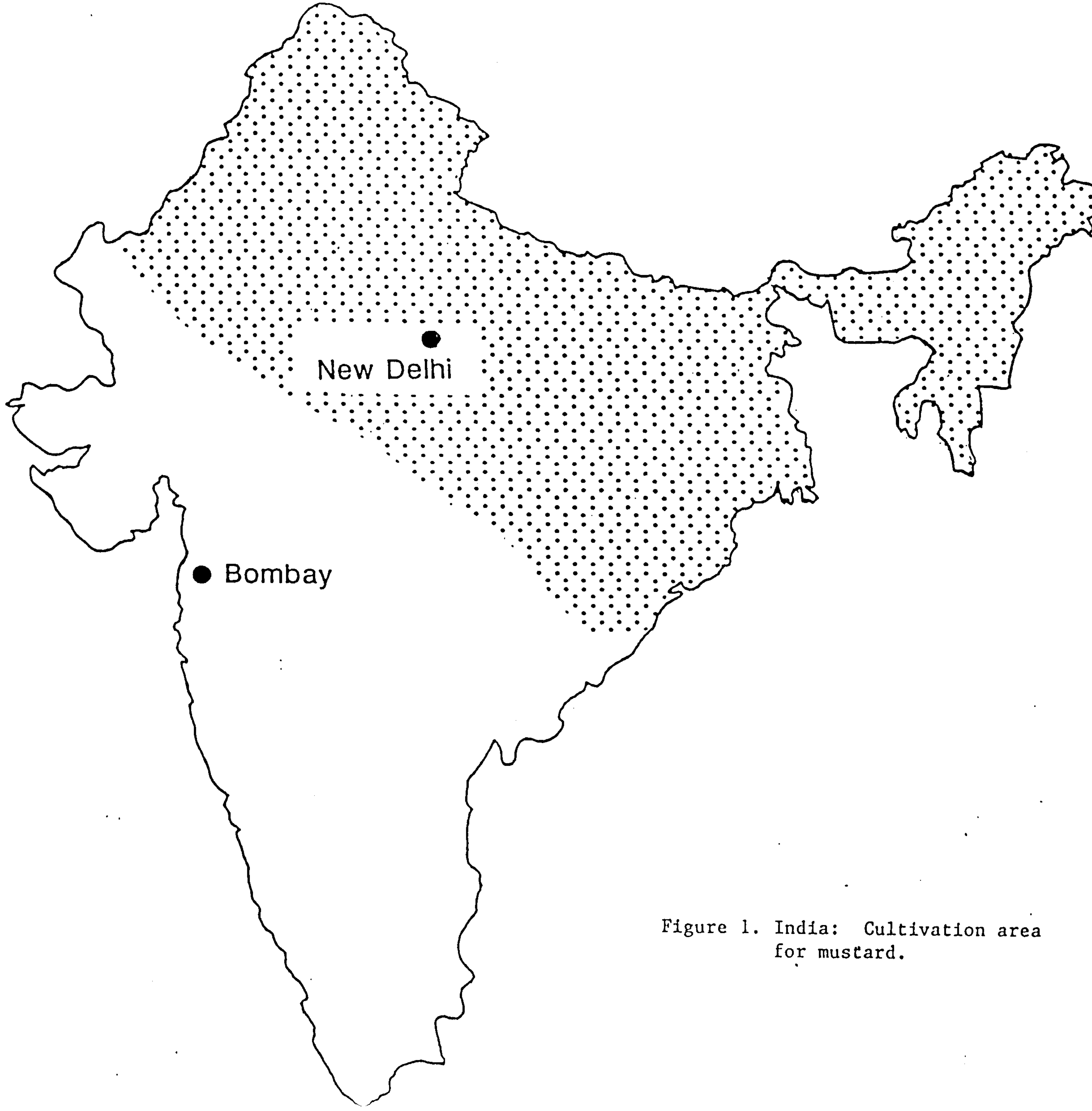

Figure 1. India: Cultivation area for mustard.

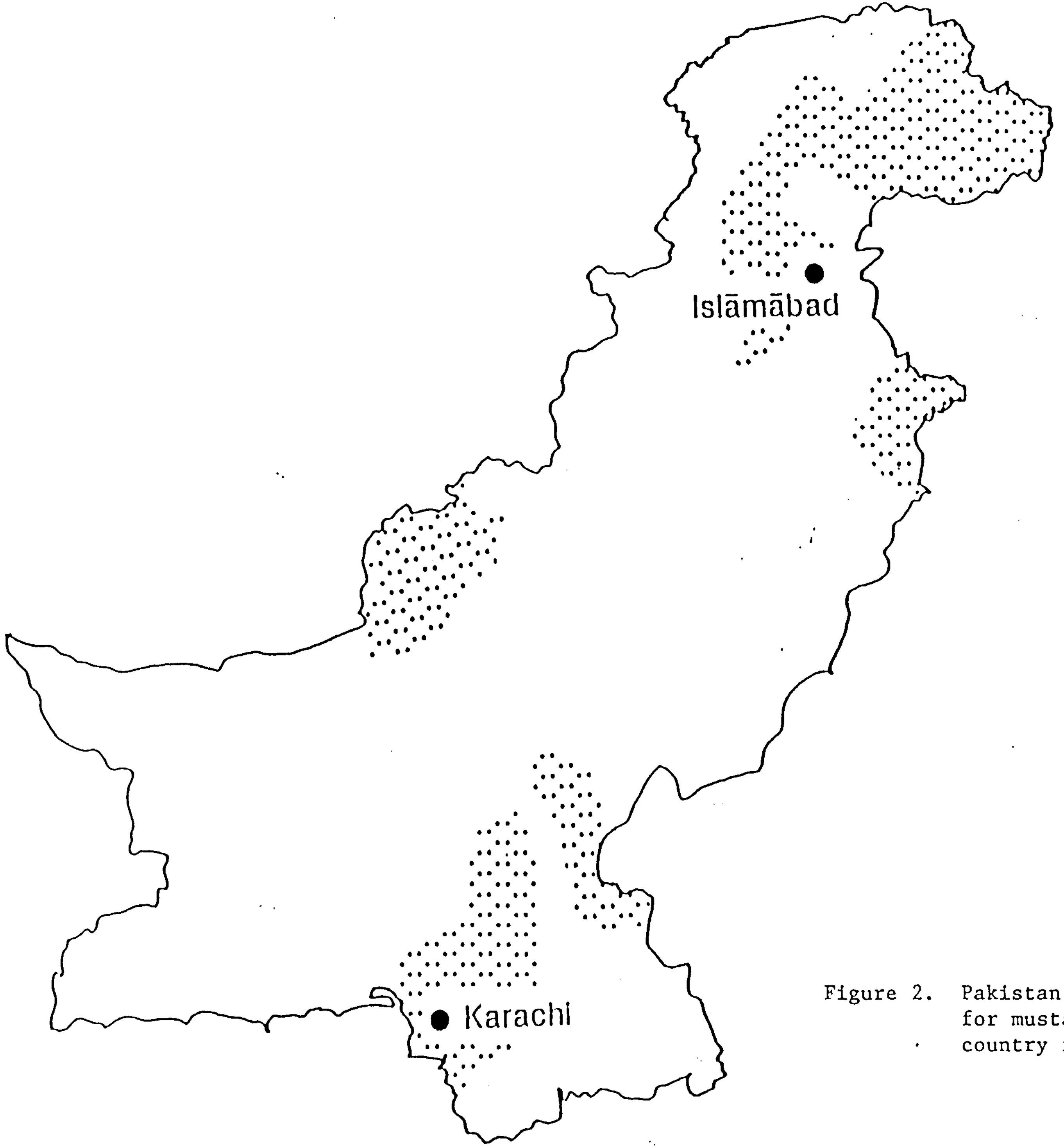

Figure 2. Pakistan: Main cultivation area for mustard (grown throughout the country for oil).